Insect- and vertebrate-selective neurotoxins from Australian urodacid and buthid scorpion venoms: lead compounds for novel biopesticides

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CERTIFICATE OF AUTHORSHIP

AND ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged in the text.

I also certify that the thesis has been written and prepared by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all sources of information and literature used are indicated in the thesis.

Harry Miller — 25.7.04

(signed) (date)

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ABSTRACT

Scorpions from nine species were collected from locations across Australia comprising four *Lychas* (Buthidae), four *Urodacus* (Urodacidae) and one *Cercophonius* (Bothriuridae). Three species had not been described previously. *Lychas* and *Urodacus* species collected in sufficient numbers were milked regularly for their venom, and characteristics of the venom determined.

Insect toxicity was qualitatively demonstrated for two *Lychas* species. Insect and vertebrate toxicity was determined for *Urodacus manicatus* and *U. hoplurus*, and both species were shown to have very low mammalian toxicity (>4mg/kg). Insect toxicity of these two venoms was estimated at 300-500μg/g by injection into crickets (*Acheta domesticus*). Venom profiles prepared by rp-HPLC revealed that *Lychas* venom was simple in composition and resembles that seen in overseas buthids. Venom composition of the *Urodacus* species was extremely complex, complicating attempts to purify individual proteins.

Toxins were purified from the venom by rp-HPLC or size-exclusion chromatography (SEC) followed by HPLC, and screened for activity in vertebrate (chick biventer cervicis) and insect bioassays. Fractions from both species showed selective excitatory toxicity towards insects and/or vertebrates. Effects on vertebrate muscle included contracture and increased stimulated twitch tension, and some fractions produced fasciculation. Further investigation was undertaken to determine the effects on ion channels in insects, as part of a search for potential biopesticide compounds.

In neurones isolated from the American cockroach terminal abdominal ganglion, the predominant effect was a block of whole-cell sodium current.

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